Adrenocorticotrophin-Releasing Hormone in Peripheral Blood: Increase during Stress

Abstract. *Significant amounts of adrenocorticotrophin-releasing hormone appear in the peripheral blood under conditions of physiological stress. Associated with the appearance of this neurohormone is an increased antidiuretic activity. The neurohormone presumably enters the general circulation by way of the portal vessels of the anterior pituitary gland.*

An ACTH-releasing hormone (ACTH-RH), also called corticotrophin-releasing factor, has been demonstrated in extracts of the median eminence and of the posterior pituitary (*1*). This neurohormone is, apparently, released into the primary plexus of the portal vessels in the median eminence and transported to the sinusoids of the anterior pituitary where it acts upon the cells which produce ACTH, causing the release of this hormone. The studies reported here indicate that the peripheral blood from animals subjected to severe physiological stresses contains ACTH-releasing and antidiuretic activity and that the hormone (or hormones) associated with this dual activity enters the general circulation by way of the portal vessels of the anterior pituitary.

The assay of ACTH-RH in plasma is, briefly, as follows. The test animal is the male rat of the Fischer strain bearing an ACTH-producing pituitary tumor (Furth's MtT F4) (*2*). A suspension of MtT F4 cells was injected into the muscles of the right hind leg of rats weighing about 200 g. Thirteen days later the animal was hypophysectomized, and 24 hours later the assay was carried out. A four-point assay with five animals for each point was used, but in order to find the optimum range of hormone activity, a six-point assay was set up. The plasma being assayed was given in doses of 0.1, 0.33, and 1.0 ml, made up to 1 ml volume with a 5 percent solution of crystalline bovine albumin. Since a stable reference standard for ACTH-RH is not available, its activity in the plasma is expressed as the amount of ACTH released by 1 ml plasma; thus the reference standard used in these experiments is ACTH.

A highly purified preparation of ACTH furnished by the Armour Laboratories had a potency of 35 U.S.P. units per milligram. The ACTH was given in doses of 33, 100, and 300 microunits (μU) in 1 ml of 5-percent albumin. The rats were anesthetized lightly with urethane (200 mg intraperitoneally). The left adrenal was first removed; the plasma was injected slowly (1 ml/min) into the abdominal aorta through a needle tied in the left common iliac artery so that the plasma was carried directly into the arterial bed of the tumor. Exactly 5 minutes after the start of the infusion, the right adrenal was removed. The adrenals were dissected, weighed, ground in saline and alcohol, and frozen within 1 minute after removal. The increase in corticosterone content of the right adrenal over that of the left in this five-minute period was a measure of the ACTH released from the tumor by the injected plasma. The rats receiving the ACTH standard were treated in exactly the same way as those given plasma except that the ACTH was injected into the jugular vein. The log of the dose of ACTH plotted against the increase in corticosterone in the right adrenal over that in the left is a linear function in the range of 33 to 300 μU. The index of precision (λ) for the assay falls in the area of 0.097.

An illustration of a routine four-point assay is shown in Fig. 1. Two groups of five rats each were given standard ACTH, 33 and 100 μU, respectively, and two groups received plasma from a pooled sample collected from stressed rats, in doses of 0.1 ml and 0.33 ml, respectively. The regression lines for the standard ACTH and the plasma are essentially parallel. By graphical interpolation the assay showed that 0.1 ml of plasma released 33 μU (1.0 ml = 330 μU) and that 0.33 ml released 120 μU (1.0 ml = 360 μU). The method used for the assay of ACTH is somewhat similar to the aforesaid method for assaying ACTH-releasing hormone except that the hypophysectomized rats are without pituitary tumors (*3*). Antidiuretic hormone was assayed by the method of Ames and van Dyke (*4*).

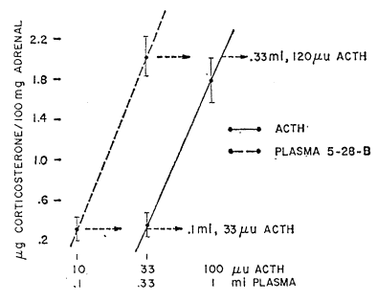

Fig. 1. Four-point assay for ACTH-RH. Plasma is from rats in experiment 1, indicated in Fig. 2. An increase of 0.3432 μg of corticosterone per 100 mg of adrenal was produced by 0.1 ml of plasma; by interpolation on the ACTH standard curve this corresponds to 33 μU of ACTH. Similarly 0.3 ml of plasma → 2.034 μg of corticosterone → 120 μU of ACTH. The mean ± S.E. is indicated by the vertical bars.

Rats were subjected to various forms of stress, and the concentration of ACTH-releasing hormone in the peripheral blood was measured. The most severe physiological stress appears to be a combination of laparotomy under ether anesthesia with rapid removal of blood from the aorta. In the first experiment (Fig. 2) 3 ml blood were withdrawn over a 1-minute period, another 3-ml withdrawal following immediately in the next 3 minutes. Blood pools were made up from ten rats in the group, the rats weighing 250 g. A control group consisted of hypophysectomized rats, 1 month after operation, which were bled from the aorta while under ether anesthesia. In the nonstressed group, blood was collected from the neck after decapitation. The second experiment (Fig. 2) was identical with the first, except that three bleedings were made. Plasma from stressed hypophysectomized rats and from nonstressed intact rats contains no ACTH-RH detectable by this method, while the blood from stressed rats shows very high titers of ACTH-releasing hormone: for example, 1 ml of plasma from the first bleeding in experiment 1 released 320 μU of ACTH, from the second bleeding, 220 μU of ACTH. Experiment 2 showed still higher titers of ACTH-RH in all three bleedings.

Since the concentration of ACTH in the blood increases under stress conditions (*5*), it is important to know the concentration of ACTH in the plasma as well as the ACTH-RH content. The amount of plasma needed for the ACTH assay is 5 to 10 times that needed for ACTH-RH assay. For that reason the ACTH assay is not done routinely. In experiment 2 (Fig. 2) plasma from the second and third bleedings had ACTH values of 22 and 18 μU/ml, respectively. It is evident that the error in the values of ACTH-RH activity, if not corrected for ACTH

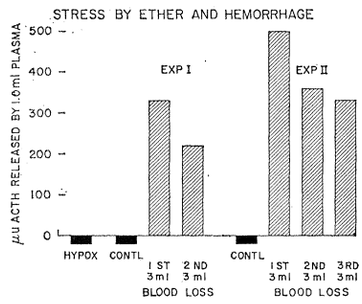

Fig. 2. The concentration of ACTH-RH in plasma of rats stressed by ether anesthesia and bleeding. The ACTH-RH activity is expressed as microunits of ACTH released by 1.0 ml of plasma.

content of plasma, is less than 10 percent.

The release of ACTH-RH into the blood during the stress of noise and air turbulence was studied. Each rat was placed in a museum jar, and a rubber hose from the compressed air line of the laboratory was directed into the jar. The animal was subjected to the noise and turbulence from the jet stream for 1 minute; a minute later the rat was decapitated, and blood was collected. Blood from the control rats was collected after decapitation with the minimum of handling. The ACTH-RH titer from this stress was equivalent to 60 μU of ACTH released by 1 ml of plasma. No ACTH-RH was detected in the plasma of the nonstressed controls. Tension on the vagus nerve as a stress stimulus causes a very marked rise of ACTH-RH in the blood. Rats had been prepared, 24 hours in advance, with black silk threads placed around both vagi and brought to the outside of the neck. The animals were subjected to tension on both vagi for 1 minute; 2 minutes later they were decapitated for blood collection. During the vagal stimulation the heart rate slowed to 60 beats per minute, and the body became limp; the heart rate returned to normal during the 2 minutes after stimulation. The ACTH-RH titer from this stress was equivalent to 300 μU of ACTH released by 1 ml of plasma. In another experiment the pain from cutting the tip of the tail of the rat did not prove to be a sufficient stress stimulus to cause detectable ACTH-RH in the plasma.

Table 1. ACTH-Releasing and antidiuretic activity in plasma of stressed rats.

Conditions	ACTH-RH activity*	ADH (μU/ml)	Notes
Dehydrated† 48 hours			
Stressed	600	300	ACTH 46 μU/ml
Non-stressed	0‡		ACTH 4 μU/ml
Dehydrated 72 hours			
Stressed	60	125	
Non-stressed	0	0	No ACTH
Dehydrated 144 hours			
Stressed	52	150	
Non-stressed	0	0	
Not dehydrated			
Stressed	220	200	ACTH 22 μU

* ACTH-RH activity expressed as microunits released by 1 ml of plasma. † Access to food, not water. ‡ No activity detected in 1 ml of plasma.

Antidiurectic activity has been estimated in a few of the plasmas assayed for ACTH-RH (Table 1). The data shown here should be considered as approximate estimates of antidiuretic hormone (ADH). Antidiuretic activity was significantly increased in the plasma from stressed animals, whether the animals had been dehydrated or allowed access to drinking water; in the nonstressed animals there was no detectable antidiuretic activity in 1 ml of plasma even after 3 and 6 days of dehydration.

An increase in antidiuretic activity in blood and urine during stress has been reported by numerous investigators. Ginsburg and Heller (*6*) have shown an increase of antidiuretic hormone as high as 1.3 mμ/ml in blood from the jugular vein. Their findings, together with ours, pose the question whether the ACTH-releasing hormone in plasma from stressed animals has antidiuretic activity, or whether there are two distinct hormones, ACTH-releasing and antidiuretic in the plasma. We have reported earlier that synthetic arginine vasopressin has the property of releasing ACTH (*7*); however, an unexplained finding has been that the degree of response in our test is the same whether the dose of vasopressin is 1.0 or 10.0 μU. Since the ACTH-releasing hormone in plasma gives a linear dose response, the plasma ACTH-RH is probably not identical with synthetic arginine vasopressin (*8*).

There is also the question of how ACTH-releasing hormone reaches the peripheral blood stream, since it is, supposedly, transported via the portal vessels from the median eminence into the sinusoids of the anterior lobe. To determine whether it escapes from the sinusoids and enters the venous outflow of the pituitary into the general circulation, the anterior lobe of rats was removed, leaving the median eminence, stalk, and posterior and intermediate lobes intact. Extirpation of the anterior lobe presumably caused the portal vessels to be blocked at a point just proximal to the site where they merge with the sinusoids of the anterior lobe. Three groups of ten rats each had the anterior lobe removed, and periods of 24 hours, 15 days, and 18 days, respectively, were allowed to elapse before the rats were stressed by ether and bleeding. In the group of rats which were carried for 18 days, water was withheld for the last 3 days. After the bleeding, the sella was examined under the dissecting microscope; and if any recognizable anterior pituitary tissue was present or if the neurohypophysis appeared damaged, the blood from that animal was discarded. The plasma from these stressed rats lacking their anterior pituitary was assayed for ACTH-RH, and in none of the plasmas from the three groups was any ACTH-RH detected. Microscopic examination of the serial sections of the sella, stalk, and hypothalamus of three rats selected at random revealed normal-appearing neurohypophysis and tuberoinfundibular and supraoptic nuclei. Thus, there seems to be substantial evidence that the access into the peripheral circulation of ACTH-RH is via an intact portal system of blood vessels.

EVELYN ANDERSON
Ames Research Center,
Moffett Field, California 94035

References and Notes

1. M. Saffran, A. V. Schally, B. G. Benfy, *Endocrinology* **57**, 439 (1955); R. Guillemin, W. R. Hearn, W. R. Cheek, D. E. Householder, *ibid.* **60**, 488 (1957).
2. J. Furth, E. L. Gadsden, K. H. Clifton, E. Anderson, *Cancer Res.* **16**, 600 (1957); J. Furth, K. H. Clifton, E. L. Godsden, R. F. Buffett, *ibid.* **16**, 608 (1957); F. E. Wherry, L. N. Trigg, R. E. Grindeland, E. Anderson, *Proc. Soc. Exp. Biol. Med.* **110**, 377 (1962).
3. J. Vernikos-Danellis, E. Anderson, L. N. Trigg, in preparation.
4. R. G. Ames and H. B. van Dyke, *Endocrinology* **50**, 350 (1952).
5. K. L. Sydnorand G. Sayers, *ibid.* **55**, 621 (1954); J. R. Hodges and J. Vernikos, *Acta Endocrinol.* **30**, 188 (1959).
6. M. Ginsburg and H. Heller, *J. Endocrinol.* **9**, 274 (1953).
7. R. E. Grindeland, F. E. Wherry, E. Anderson, *Proc. Soc. Exp. Biol. Med.* **110**, 377 (1962).
8. Experiments carried out since this report was submitted for publication suggest that the ACTH-releasing and antidiuretic activities involve separate hormones. In rats with the posterior pituitary lobe removed, the anterior pituitary and the hypophyseal portal circulation being left intact, there is no detectable ADH in the plasma after stress, but there is a high concentration of ACTH-RH. With the anterior pituitary removed and the entire neurohypophysis being left intact there is an elevated concentration of ADH in the plasma after stress but no detectable ACTH-RH.
9. We thank Dr. J. D. Fisher of Armour and Company for a generous supply of ACTH Standard.

8 December 1965